Co-Agglutination test as a diagnostic method for *Trichomonas vaginalis* infection in women

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By

MONA MOHAMMED ANWAR

M.B., B.Ch.
Demonstrator of Parasitology
Faculty of Medicine, Ain Shams University

Supervisors

Prof. Dr. IMAN MOAWAD ABD EL SALAM

Professor of Parasitology
Faculty of Medicine, Ain Shams University

Dr. FAIZA SAYED MOHAMED HABIB

Assistant Professor of Parasitology
Faculty of Medicine, Ain Shams University

Dr. HANAN HUSSEIN KAMEL

Assistant Professor of Parasitology
Faculty of Medicine, Ain Shams University

Parasitology Department
Faculty of Medicine, Ain Shams University
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استخدام اختبار التلازن المختلط كطريقة لتشخيص الإصابة بطفيل الترايكوموناس فاجيناليس في النساء

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مقدمه

ط/ مني محمد أنور أحمد
معيدة بقسم الطفيليات، كلية الطب، جامعة عين شمس

المشرفين

الأستاذة الدكتورة/ ايمان معوض عبد السلام
أستاذ علم الطفيليات، كلية الطب، جامعة عين شمس

دكتورة/ فايزة سيد محمد حبيب
أستاذ مساعد علم الطفيليات، كلية الطب، جامعة عين شمس

دكتورة/ حنان حسين كامل
أستاذ مساعد علم الطفيليات، كلية الطب، جامعة عين شمس

قسم الطفيليات
كلية الطب، جامعة عين شمس
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INTRODUCTION

Trichomoniasis, an extremely common infection worldwide, characterized by vaginitis in female and urethritis in male, is caused by the protozoan *Trichomonas vaginalis*. The World Health Organization has estimated that this infection accounts for almost half of all curable infections worldwide (**Schwebke and Burgess, 2004)**.

Women who are symptomatic from trichomoniasis complain of vaginal discharge, pruritis, and irritation. Nearly half of all women with *T. vaginalis* are asymptomatic **(Fouts and Kraus., 1980).** The prevalence and spectrum of disease in males are less well characterized; the infection appears to be usually asymptomatic, but it has been suggested as an increasingly important cause of non-gonococcal urethritis **(Laga *et al.*, 1993).**

Long considered a "minor" Sexually Transmitted Disease (STD) with few associated complications, infection with *T. vaginalis* has recently been implicated as a cause of some important public health problems. In a large multicenter study, after adjusting for demographic, behavioral, and microbiological variables, *T. vaginalis* was significantly associated with low birth weight, premature rupture of membranes, and preterm delivery **(Cotch *et al.*, 1997).** In

addition, transmission of HIV (Human Immune defeciency Virus) is reported to be enhanced in men by co-infection with *T. vaginalis*. **(Price et al., 2003).**

The most common mean of diagnosis is visualization of the motile trichomonads in a saline preparation of the vaginal fluid. This must be performed within 10 to 20 minute of collection of the sample, or the organisms will lose viability. Although quick and inexpensive, the test has limited sensitivity, ranging from 60 to 70% **(Garcia, 2003).** Currently, the "gold standard" for the diagnosis of trichomoniasis is culture. Traditionally, this has been accomplished through cultivation in Diamond's medium, which is not widely available and thus was used mainly for research purposes. Results of the culture test are available in 2 to 5 days **(Garcia, 2003).** Permanent stains (methylene blue and Giemsa stains), fluorescent stains (acridine orange) are other useful diagnostic methods **(Garcia, 2003).** Antigen detection test for the diagnosis of trichomoniasis in women has been licensed. The enzyme-linked immunosorbent assay demonstrated good sensitivity and specificity **(Alderete and Garza, 1984, Azab *et al.,* 1992 and Azab *et al.,* 1999)**. Examination of urine sediments for the presence of trophozoites may be useful **(Garcia, 2003)**. In general, these techniques are time-consuming and are limited by the specificity. Thus other tests that is rapid, simple to process, inexpensive and at the same

time highly sensitive and highly specific is needed (**Schwebke and Burgess, 2004)**.

The co-agglutination (Co-A) test is a novel immunological method, it is often used to confirm the identification of bacterial colonies in culture plates. It is similar to the Latex Agglutination (LA) technique for detecting antigen. Protein A, a uniformly distributed cell wall component of *Staphylococcus aureus*, is able to bind to the Fc region of most IgG isotype antibodies leaving the Fab region free to interact with antigens present in the applied specimens. The visible agglutination of the *S. aureus* particles indicates the antigen-antibody reaction. **(Koivunen and Krogsrud, 2006).** The test has already found some applications in parasitology; It was used to detect *parasite* antigen in urine in toxoplasmosis (**Facado *et al*.,1990** and **1992)**, in hydatid fluid in hydatidosis (**Shariff and Parija, 1993)**, in blood in invasive amoebiasis **(Karki and Parija, 1999)**, in stools in trichinosis **(Boulos *et al.,* 2001),** in blood in cysticercosis **(Parija and Reddy, 2006)** and in blood, stool and water in cryptosporidiosis **(Michel *et al*., 2000).**

REVIEW OF LITERATURE

I- TRICHOMONAS VAGINALIS:

History and Taxonomic Classification:

T.vaginalis, a protozoan parasite, was first identified by the Parisian physician Alexandre Donne' in 1836, he described its 'undulating motion' and 'whiplike tail' in the purulent, frothy leucorrhea of women presenting with vaginal discharge and genital irritation. He gave the name 'Trico-Monas' to these organisms because of morphological similarities to two other protozoa known at that time, 'tricodes' and 'monas' (**Jamali *et al.,* 2006**). However, the disease was first described as a clinical entity by **Höhne** in **1916**.The taxonomic position of *Trichomonas vaginalis* is based on the classification scheme by **Dyer (1990).**

- Phylum : Zoomastigina.
- Class : Parabasalia.
- Order : Trichomonadida (**Kirby, 1947** emend**. Honigberg, 1974**).
- Family : Trichomonadidae (**Wenyon, 1926**).
- Genus : *Trichomonas* (**Donne', 1837**).
- Species : *vaginalis* (**Donne', 1836**).

Morphology:

Trichomonas vaginalis has only the trophozoite stage in its life cycle and does not form cyst (**Petrin *et al.*, 1998, Jamali *et al.*, 2006 and Adegbaju and Morenikeji, 2008).** It varies in size and shape, with the average length and width being 7-23 µm and 5-15 µm respectively **(Garcia, 2007).** Physiochemical conditions do alter the appearance of the parasite. Using scanning electron microscopy, **Ryu and Min (2006)** compared the sizes of trophozoites cultivated in trypticase-yeast extract-maltose (TYM) medium with those of trophozoites freshly isolated from vaginitis patients, and it was found that the lengths of the axostyles and flagella of trophozoites freshly isolated from trichomoniasis patients were significantly longer. It is believed that trophozoite shape and length may be changed by environmental conditions, i.e. *in vivo*, in the vagina and *in vitro*. In axenic culture, the shape of the protozoan tends to be more uniform, i.e. pear shaped or oval but the parasite takes on a more amoeboid appearance when attached to vaginal epithelial cells (**Petrin *et al.*, 1998, Rughooputh and Greenwell, 2006 and Garcia, 2007**). It is a flagellated protozoan possessing five flagella, four of which are located on its anterior portion. The fifth flagellum is incorporated within an undulating membrane supported by a slender non contractile costa (**Jamali *et al.*, 2006**). The undulating membrane is about 2/3 or 1/2 of the body length

with no free flagellum (**Karaman *et al.,* 2008**). The functionality of the fifth flagellum is not known (**Rayan and Ray, 2004**). The flagella and the undulating membrane give this parasite a characteristic quivering movement. The undulating membrane and the costa originate in the kinetosomal complex at the anterior of the parasite **(Burgess, 2005).** The nucleus in *T.vaginalis* is usually elongated **(Ash and Orihel, 2007)**, prominent **(Burgess, 2005)**, located at its anterior portion, and is surrounded by a porous nuclear envelope (**Petrin *et al.,*1998)**, with evenly distributed chromatin **(Garcia, 2007).** A rigid, rod –like structure, called an axostyle (**Schwebke and Burgess, 2004 and Burgess, 2005)**, 3-14 μm in length **(Ryu and Min, 2006)** commences at the nucleus and bisects the protozoan longitudinally. It protrudes through the posterior end of the parasite, terminating in a sharp point **(Ash and Orihel, 2007)**. The axostyle is thought to anchor the parasite to vaginal epithelial cells and may also cause the tissue damage noted in trichomoniasis **(Rayan and Ray, 2004).** A parabasal body, a single and V-shaped structure, having a filament associated with it is present **(Adegbaju and Morenikeji, 2008).** A cytostome, a conspicuous cleft opposite the undulating membrane, is much less conspicuous in *T.vaginalis* in comparison to other trichomonad **(Petrin *et al*., 1998).**

The cytoplasm contains a remarkably large amount of granules arranged in two sets: paracostal and paraxostylar. The latter set is arranged along the axostyle in three parallel rows, which is a distinguishing feature of *T. vaginalis*. They are seen as chromatic granules by light microscopy and as osmophilic granules by electron microscopy **(Petrin *et al.*, 1998 and Schwebke and Burgess, 2004)**. Biochemical studies have shown that these bodies serve roughly the same function as mitochondria since they contain enzymes of the Kreb's cycle **(Marquardt *et al*., 2003 and Adegbaju and Morenikeji, 2008).** They are termed hydrogenosomes **(Petrin et al., 1998 and Marquardt *et al*., 2003)** because the end product of carbohydrate metabolism is molecular hydrogen rather than water as in other eukaryotes **(Marquardt *et al*., 2003).** Glycogen granules also present in *T. vaginalis* and can be observed by transmission electron microscopy **(Petrin *et al*., 1998 and Sood and Kapil, 2008).**

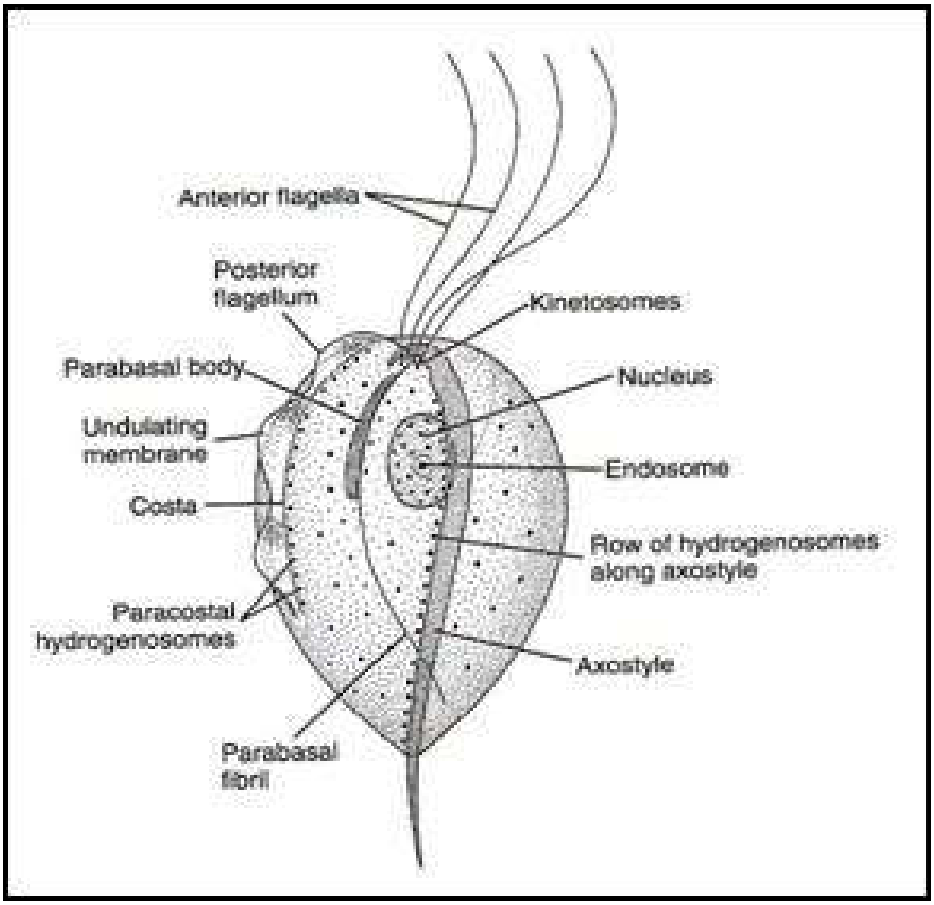


Fig. (1): Diagram of *Trichomonas vaginalis* trophozoite. (bioweb.uwlux.edu/…/strous_mary/basic_info.htm).

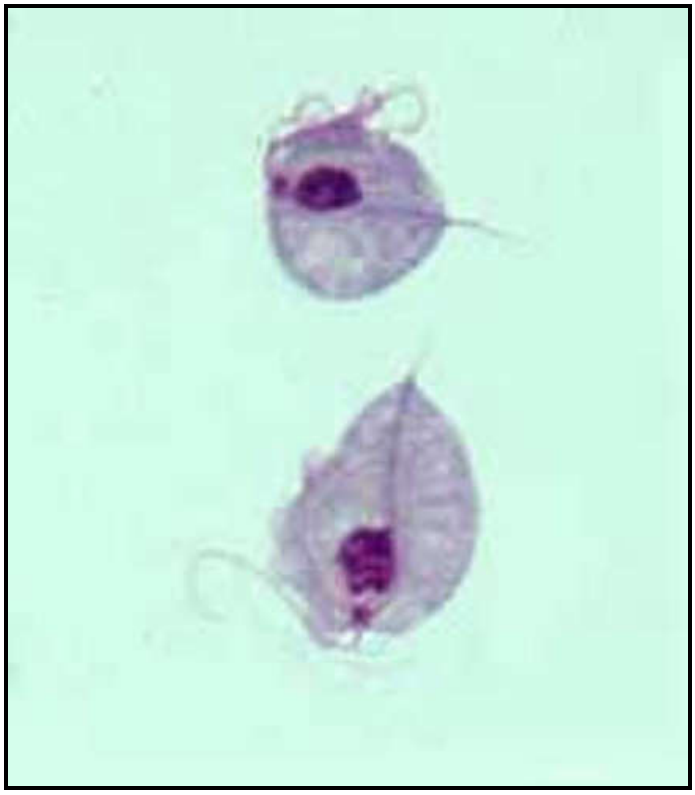


Fig. (2): *T.vaginalis in vitro* culture stained with Giemsa stain. (http:commons.wikimedia.org/wiki/Image:*Tichomonas*_Giemsa_DPDx.JP.)

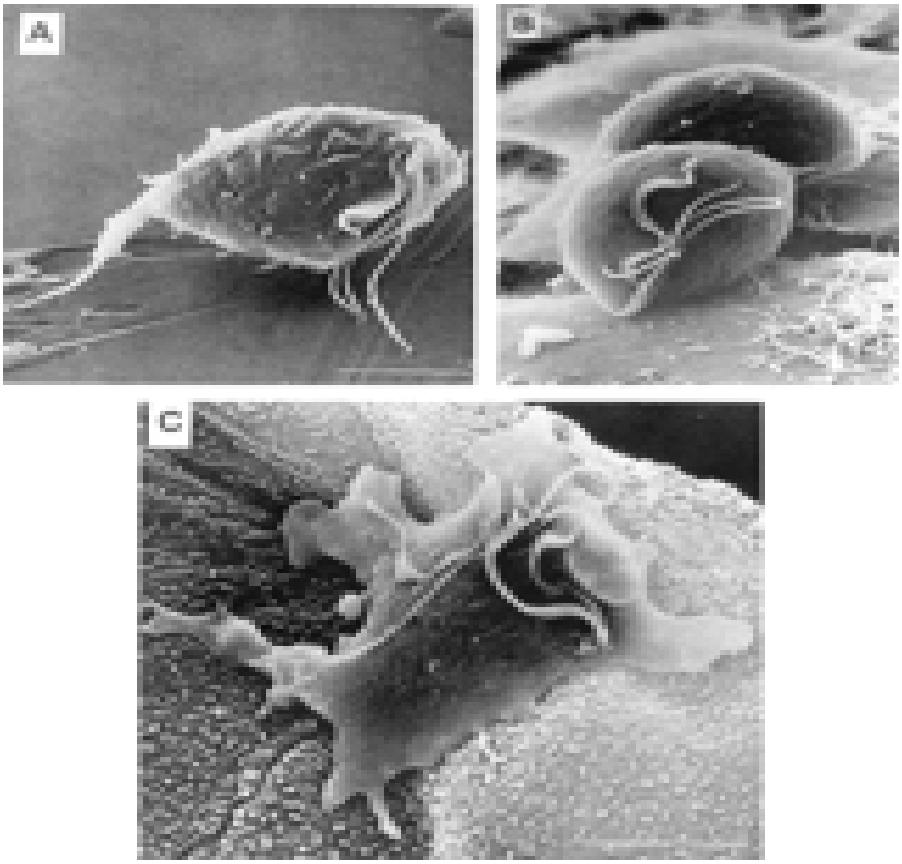


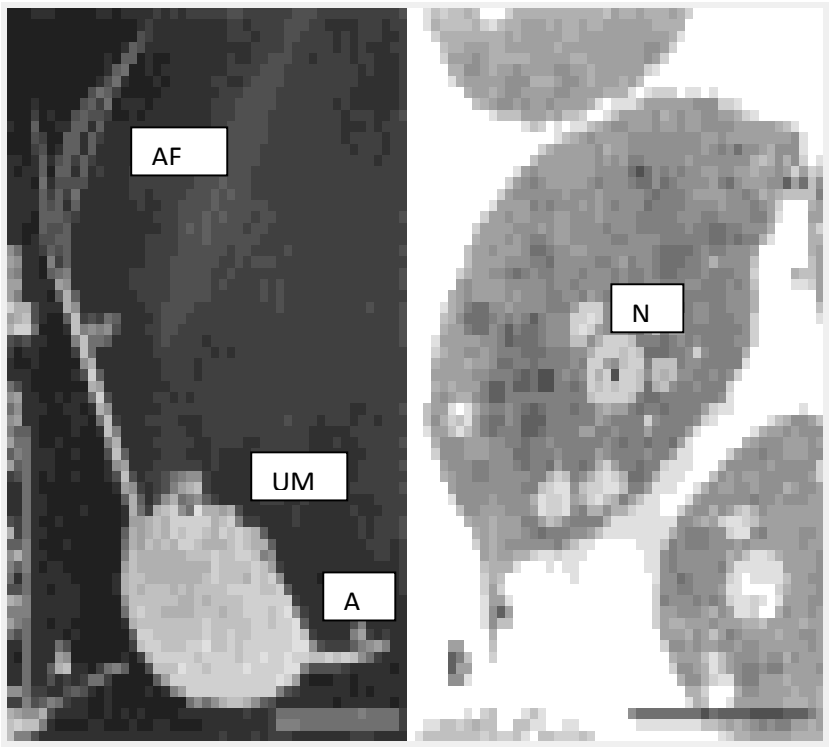
Fig. (3): Scanning electron microscopy for *T.vaginalis* **(Petrin *et al.* 1998).**

(A) *T. vaginalis* parasite as seen in broth culture. The axostyle, undulating membrane, and flagella are clearly visible.

(B) *T. vaginalis* on the surface of a vaginal epithelial cell prior to ameboid transformation.

(C) Ameboid morphology of *T. vaginalis* as seen in cell culture. Note that the side opposite the undulating membrane adheres to the vaginal epithelial cell.

Bars, 5 μm.



(A) **(B)**

Fig. (4): A. Trophozoites of *T. vaginalis* showing 4 anterior flagella (AF), an undulating membrane (UM) with posterior flagellum, and an axostyle (A).

B. This transmission electron microscope photograph illustrates a nucleus (N), hydrogenosomes (H), anterior flagella, many vacuoles (V) and an axostyle. Bar = 5 μm **(Ryu and Min, 2006).**

Habitat, Reproduction and Life Cycle:

The normal habitats of *T.vaginalis* are the mucosal surface of the human vagina, the prostate gland and seminal vesicle **(Ash and Orihel, 2007).** In the female host, the organism involves squamous rather than columnar epithelium, only rarely it can be isolated from the endocervix, but the urethra is involved in 90% of infected cases **(Cook, 2003).** In the infected male, it has been demonstrated in the epididymis and prostate, and occasionally causes non gonococcal urethritis (NGU) **(Cook, 2003).**

The life cycle of *T.vaginalis* is simple in that the trophozoite is transmitted through coitus and no cyst form is known **(Schwebke and Burgess, 2004).** It divides by longitudinal binary fission, without the disappearance of the nuclear membrane. This event begins with the duplication of selected locomotor organelles, which is followed by the development of two attractophores flanking either side of the nucleus, which become the poles for division. From the attractophores develop chromosomal microtubules, which extend toward and into the nucleus, attaching to the centromeres of the chromosomes. Also extended between the attractophores is an extranuclear spindle, called the paradesmose. This extranuclear spindle elongates, and the daughter cells separate. Each daughter cell then produces any missing organelles **(Petrin *et al.*, 1998).**

When environmental conditions are unfavorable, the organisms may tend to round up and internalize the flagella. Although it has been suggested that this may be a preliminary or pseudocyst form, it is probably just a degenerating trophozoite form. The life cycle has been described but remains poorly understood. In growth phase culture, large, round forms of the organism are seen, some without flagella, some with flagella and dividing nucleus, and some with flagella and multiple nuclei. Although there are different opinions about these forms, they are probably developmental stages that appear prior to the typical mononuclear flagellates that generally reproduce by longitudinal binary fission **(Petrin *et al.*, 1998 and Garcia, 2007).**